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Muscle glycogen depletion during exercise at 9° C and 21° C

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Summary. This study compared glycogen depletion in active skeletal muscle after light and moderate exercise in both cold and comfortable ambient conditions. Twelve male subjects (Ss) were divided into two groups equally matched for the submaximal exercise intensity corresponding to a blood lactate concentration of 4 mM (W4) during cycle exercise. On two separate days Ss rested for 30 min at ambient temperatures of either 9° C or 21° C, with the order of temperature exposure being counter-balanced among Ss. Following rest a tissue specimen was obtained from the m. vastus lateralis with the needle biopsy technique. Six Ss then exercised on a cycle ergometer for 30 min at 30% W4 (range = 50–65 W) while the remaining group exercised at 60% W4 (range = 85–120 W). Another biopsy was taken immediately after exercise and both samples were assayed for glycogen content. Identical procedures were repeated for the second environmental exposure. No significant glycogen depletion was observed in the Ss exercising at 30% W4 in 21° C, but a 23% decrease ($p = 0.04$) was observed when the same exercise was performed at 9° C. A 22% decrease ($p = 0.002$) in glycogen occurred in the 60% W4 group at 21° C, which was not significantly different from that observed during the same exercise at 9° C. The results suggest that muscle substrate utilization is increased during light exercise in a cold environment as compared to similar exercise at a comfortable temperature, probably due to shivering thermogenesis. Heat produced with higher exercise intensities seems to be sufficient to prevent shivering and the accompanying glycogenolysis.

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Introduction

Increases in skeletal muscle electromyographic (EMG) activity and whole body oxygen consumption ($\dot{V}_{O_2}$) have been observed concomitant with observable shivering when man is exposed to cold (Hong and Nadel 1979; Nadel et al. 1973; Pugh 1967). These findings have implications for exercise since $\dot{V}_{O_2}$ is increased during light exercise in the cold as compared to the same exercise performed at a comfortable ambient temperature. This has been attributed to the increased metabolic demand induced by shivering (Burton and Edholm 1955; Horvath 1982). However, with increasing exercise intensity observable shivering decreases and is eventually abolished as the heat produced with exercise replaces the heat generated by shivering (Hong and Nadel 1979; Pugh 1967; Strømme et al. 1963).

Since shivering is an involuntary increase in contractile activity, it seems reasonable to speculate that the uptake of circulating substrates and/or utilization of endogenous intramuscular substrates is greater than in the absence of shivering. Although animal studies suggest that muscle glycogenolysis is increased in the cold (LeBlanc and Labrie 1981; Sobolova 1969), no direct measurements have been reported of glycogen utilization in human subjects during light exercise with whole body cold exposure. The specific purpose of the present study, therefore, was to compare intramuscular glycogen utilization during light and moderate intensity exercise at ambient temperatures of 9° C and 21° C. The experimental hypo-

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thesis was that more glycogen would be depleted during light exercise in the cold than at 21° C; since shivering is abolished during high intensity exercise it was thought that this would be reflected in muscle glycogenolysis which would be similar at 9° C and 21° C during higher intensity exercise.

Methods

Subjects. Twelve male subjects volunteered to participate after being informed of the details of the experimental protocol and the associated risks and discomforts. The protocol was approved by the Institute's human ethics committee and written informed consent was obtained from all subjects.

Evaluation of aerobic capacity. On the first visit an exercise test was performed on an electrically braked cycle ergometer (Collins Pedal-Mate) to determine the power output at a blood lactate concentration of 4 mmol L^{-1} (W4), as described previously for the onset of blood lactate accumulation (Jacobs 1981). Exercise began at 50 W with 50 W stepwise increments at 4 min intervals. During the final 30 s at each power output blood was sampled (25 µl) from the finger-tip for lactate determination (Maughan 1982). Exercise was halted when the subjects rated their perceived leg exertion (RPE) as 16 on the Borg scale (Borg 1970). W4 was interpolated from a plot of lactate concentration against power output.

Percent body fat was calculated from body density determination by hydrostatic weighing (Brozek et al. 1963). Residual volume was measured with the nitrogen washout technique using a pulmonary testing system (Hewlett Packard 47804S).

Environmental exposures. The subjects were divided into two groups equally matched for the W4 values. The low intensity (LI) group exercised at 30% W4 and the moderate intensity (MI) group exercised at 60% W4 during the subsequent trials. Each subject was exposed to both a 9° C and a 21° C environmental condition. The order of exposures was counter-balanced among subjects with two to three days between trials. On the day of the exposure the subjects reported to the laboratory in a 12 h post absorptive state. A rectal thermistor was inserted 15 cm beyond the anal sphincter. For both environmental conditions the subject, clad in cotton shorts, socks and running shoes, entered the environmental chamber and lay quietly in the supine position for 30 min on a rope mesh cot. They were instructed to lie with their arms away from their sides and with their legs spread apart. During the final 10 min of the rest period rectal temperature (Tre), oxygen uptake ($\dot{V}_{O_2}$) and the respiratory exchange ratio (RER), i.e. $\dot{V}C_{O_2}/\dot{V}_{O_2}$, were determined with a metabolic cart (Jaeger Ergo-Oxyscreen). Six subjects then exercised at 30% W4, the other six at 60% W4 for 30 min and the following variables were measured and recorded each tenth minute: Tre, heart rate, RPE, $\dot{V}_{O_2}$ and RER.

Blood and muscle samples. Blood and muscle tissue were sampled on both trials after the 30 min rest and immediately after exercise. Blood was sampled with stasis from a superficial arm vein; the samples were prepared and stored to enable subsequent assays for serum free fatty acids (FFA) (Smith 1975), plasma glucose (Bondar and Mead 1974) and lactate (Sigma 1977). The muscle tissue specimen was obtained from the m. vastus lateralis with the needle biopsy technique (Bergström 1962). One part of the pre-exercise specimen was immediately frozen in liquid nitrogen and stored at − 80° C. It was freeze dried and the content of glycogen was assayed enzymatically after acid hydrolysis (Karlsson 1971) and is expressed per kg dry muscle weight. The remainder of the tissue was used for the estimation of muscle fiber type composition based on a modified myofibrillar ATPase histochemical staining after alkaline preincubation (Guth and Samaha 1970).

Statistics. The BMDP Statistical Software Package (Dixon and Brown 1979) was used for all calculations. The significance of differences between means was set at the 0.05 level and was calculated with Student's t-test for either paired (intra-group comparisons) or independent (inter-group) comparisons. Data are expressed as means ± SD unless otherwise stated.

Results

The physical characteristics, calculated W4 values and the environmental exercise power outputs of the two groups of subjects are listed in Table 1. The LI group (which exercised at 30% W4) was taller than the MI group (which exercised at 60% W4). They also tended to be heavier and fatter than the MI group although these latter differences were not significant ($p = 0.13$). Muscle fiber type frequency in the vastus lateralis was similar in both groups.

Table 1. Physical characteristics, power outputs at 4 mmol L^{-1} lactate (W4) and the 30 min exercise intensities (mean ± SD) for the low intensity (LI, n = 6) and moderate intensity (MI, n = 6) groups

Variable	LI	MI
Age, yrs	22.3 ± 2.6	20.3 ± 1.4
Height, cm	180 ± 7	172 ± 6
Weight, kg	79.5 ± 11.9	69.8 ± 9.1
% fat	15.5 ± 6.1	11.7 ± 4.1
% slow twitch fibers	38 ± 6	38 ± 7
W4, W	179 ± 26	171 ± 23
30 min power output, W	55 ± 6	103 ± 14

As shown in Fig. 1, no significant glycogen depletion was observed in the LI group at 21° C (327 ± 60 vs. 319 ± 44 mmol glucose units · kg^{-1}), but a 23% decrease occurred when the same exercise was performed at 9° C (376 ± 50 vs. 289 ± 76 mmol glucose units · kg^{-1}). It is noteworthy that the pre-exercise glycogen levels differred in the LI group ($p < 0.01$) but not in the MI group. Post exercise glycogen levels differred between conditions only for the LI group ($p < 0.05$). A 22% decrease in glycogen levels occurred in the MI group at 21° C (384 ± 57 vs. 300 ± 47 mmol glucose units · kg^{-1}) which was similar to the decrease observed at 9° C (361 ± 69 vs. 297 ± 75 mmol glucose units · kg^{-1}).

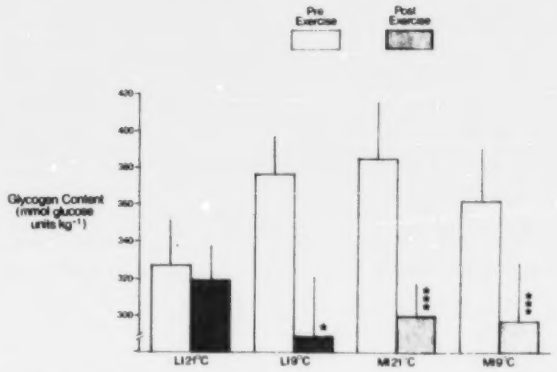

Fig. 1. Intramuscular glycogen concentrations before and after exercise at 9°C and 21°C. The low intensity group (LI) exercised at 30% and the moderate intensity group (MI) exercised at 60% of the power output which elicited a 4 mmol L^{-1} blood lactate concentration. Data are expressed as mean ±SEM. * $p<0.05$; *** $p<0.001$

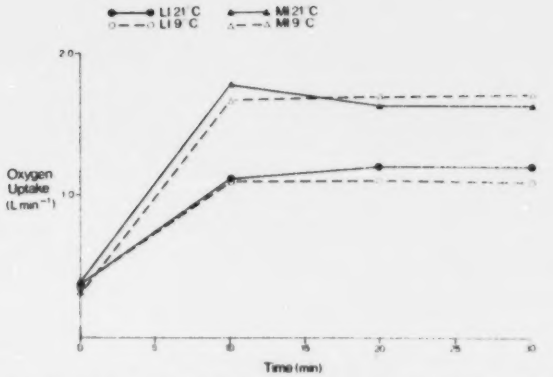

Fig. 2. Oxygen uptake during 30 min of exercise at two environmental temperatures for the low intensity (LI) and the moderate intensity (MI) groups

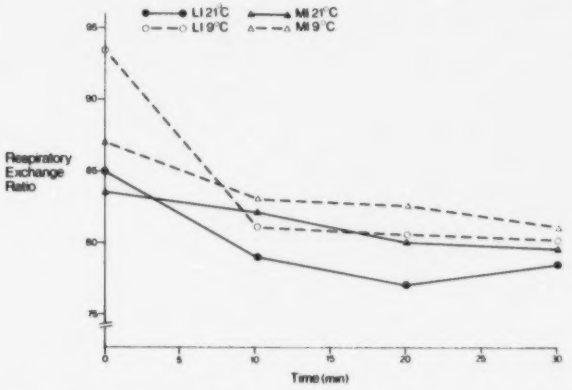

Fig. 3. Respiratory exchange ratio during 30 min of exercise at two environmental temperatures for the low intensity (LI) and moderate intensity (MI) groups

The $\dot{V}_{O_2}$ and RER during exercise in the two environmental conditions are depicted in Figs. 2 and 3. It should be noted that the $\dot{V}_{O_2}$ was significantly higher at rest ($p = 0.04$) at 9°C for the MI group only. No other differences within-groups in V_{O_2} were observed across trials. The RER tended to be higher at rest and during exercise in the cold for both groups although this difference did not achieve statistical significance.

The HR for the LI group during the last min of exercise averaged 100±10 and 98±10 beats·min^{-1} at 21°C and 9°C, respectively. The corresponding values for the MI group were 127±5 and 120±12 for the 21°C and 9°C trials. The differences between exposures were not significant for either group. The RPE values during the last min of exercise were 7.9±1.4 at 21°C and 7.3±1.0 at 9°C for the LI group, and 9.7±2.0 at 21°C and 10.3±1.6 at 9°C for the MI group; the difference between trials was not significant for either group.

The Tre increased after 10 min of exercise in both groups (Fig. 4). Within each group there was no difference between environmental exposures in the Tre values at any time during exercise. Since all subjects were exposed to similar condi-

Table 2. Blood metabolite concentrations (mean ±SD). * Indicates a significant difference ($p<0.05$) from the pre-value

Variable	Group	21°C		9°C	
		pre	post	pre	post
Lactate	LI	1.14±0.29	1.30±0.79	1.90±1.08	1.49±0.80
mmol L^{-1}	MI	0.89±0.16	1.19±0.27*	1.21±0.50	2.04±1.13
Glucose	LI	5.79±0.38	5.89±0.27	5.51±0.21	5.80±0.41
mmol L^{-1}	MI	5.99±0.28	5.81±0.43	5.83±0.48	5.65±0.23
FFA	LI	671±240	676±248	569±287	654±222
µmol L^{-1}	MI	654±141	738±248	786±300	786±218

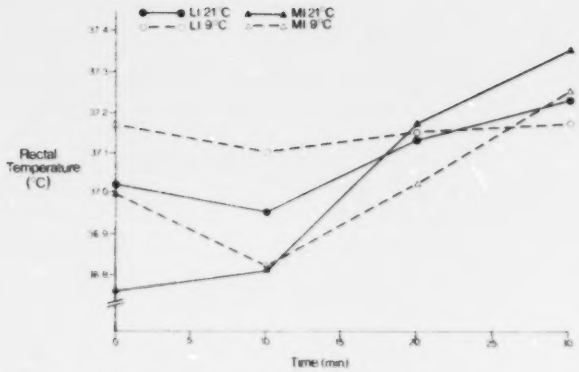

Fig. 4. Rectal temperature during 30 min of exercise at two environmental temperatures for the low intensity (LI) and the moderate intensity (MI) groups

tions until exercise commenced, the responses to the 30 min cold exposure can be examined for the subjects as a single group. When considered in this manner, the subjects had a higher Tre after 30 min of rest at 9° C than at 21° C ($p = 0.004$).

The blood metabolite concentrations were not significantly changed with exercise in either group with the exception of the slight increase ($p = 0.039$) observed in lactate for the MI group at 21° C (Table 3). When all subjects were considered as a single group, resting concentrations of glucose were lower ($p = 0.044$) and lactate was higher ($p = 0.017$) at 9° C than at 21° C.

Discussion

Very little research has been reported concerning the effects of acute cold exposure on muscle substrate utilization in humans. This is surprising because it is generally accepted that shivering is the prime source of thermogenesis during cold exposure in man (Burton and Edholm 1955). This assumption served as the basis for the present experimental hypothesis which was accepted in light of our main finding that more skeletal muscle glycogen is depleted when light exercise is performed at 9° C than when the same exercise is performed at 21° C. It was also demonstrated that higher intensity exercise abolished this difference in glycogen depletion patterns. This latter observation is consistent with findings that increasing exercise intensity inhibits the shivering response because of either nonspecific arousal which accompanies increased muscular activity, and/or the increased heat production associated with the increased muscular work (Hong and Nadel 1979; Pugh 1967).

We cannot exclude entirely the possibility that the statistically significant difference between pre-exercise glycogen concentrations for the LI group at 21° C and 9° C may have influenced substrate utilization during the subsequent exercise. Sherman et al. (1981) demonstrated that more glycogen depletion occurs for a given amount of exercise after a few days of a carbohydrate rich diet when pre-exercise glycogen levels are elevated than after a low carbohydrate diet when resting glycogen levels are lower prior to exercise. However, both LI pre-exercise glycogen levels in the present study fall well within the range of normal concentrations to be expected after an uncontrolled mixed diet and should not have influenced subsequent glycogen depletion during exercise (Bergström et al. 1967; Sherman et al. 1981). Moreover, no significant relationship was obvious in the present study when pre-exercise glycogen levels were plotted against the observed depletion for either group or condition.

Fink et al. (1975) reported greater glycogen depletion during exercise in the heat than in the cold; no comparison was made to a comfortable ambient temperature. Moreover, exercise intensity in that study was 70—85% $\dot{V}_{O_2max}$, an intensity which would certainly override any shivering threshold (Hong and Nadel 1979), particularly since exercise began immediately after entering the environmental chamber without any time to respond to the cold stimulus per se.

Observable shivering ceased in the subjects in the present study once they began to exercise. This does not necessarily mean that the involuntary muscle activity associated with shivering halted; Pozos (1981) observed increased EMG activity in individuals without observable shivering. This "low shivering" was attributed to an increased muscle tone (Pozos 1981). Such "low shivering" may have been maintained during the LI exercise in the present study, thereby demanding increased substrate utilization during exercise in the cold compared to the 21° C exposure.

The demand for substrate could be met by greater muscle uptake of circulating substrates, a greater utilization of intramuscular substrates, or a combination of these mechanisms. Since only absolute concentrations of selected blood substrates were measured no conclusions can be made regarding their contribution to the increased metabolic demand. The fact that only minimal changes were observed in blood concentrations emphasizes the possible errors in interpreting circulating substrates as being representative of intramuscular events.

It was expected that the greater glycogenolysis in the LI group at 9° C would be accompanied by a higher $\dot{V}_{O_2}$ and/or RER during exercise when compared to the 21° C trial. This was not the case and cannot be explained based on the data at hand. The $\dot{V}_{O_2}$ data for both exercise groups and temperature conditions are similar to those reported by Strømme et al. (1963), and are therefore not unreasonable. Other studies, however, demonstrated elevated $\dot{V}_{O_2}$ during light exercise in the cold (Hong and Nadel 1979; Horvath 1982; Pugh 1967). The fact that our subjects rested only for 30 min probably diminished the thermoregulatory stimuli associated with decreased skin temperature which reach a steady state after about 1 h of exposure to 9—10° C air (Hong and Nadel 1979).

Another explanation for the seemingly conflicting glycogen and $\dot{V}_{O_2}$ results may be attributed to our procedure of measuring $\dot{V}_{O_2}$ and RER during the final 1—2 min of each 10 min exercise interval. An increase in non-specific arousal stimuli inhibits shivering (Shimada and Stitt 1983). Since shivering occurs intermittently at air temperatures of 9° C (Horvath 1982), it is possible that the extra arousal caused by inserting the mouthpiece, placing the noseclips, and the knowledge that $\dot{V}_{O_2}$ was being measured may have suppressed or totally inhibited shivering during the $\dot{V}_{O_2}$ sampling period. A preferable procedure, recommended for future studies, would be to monitor $\dot{V}_{O_2}$ continously throughout the rest and exercise period. In light of these confounding effects on $\dot{V}_{O_2}$, one wonders whether the measurement of muscle glycogen may not be a more reliable indicator of the extent of cold stress and shivering than is $\dot{V}_{O_2}$.

In conclusion, the present study demonstrates that intramuscular glycogen utilization is increased when light exercise is performed in the cold. The possible implications of these findings may extend to include the nutritional requirements and relative carbohydrate content of diets for prolonged periods of activity in a cold environment.

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